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Vol. XXVII October, 1943 No. 4

STUDIES ON TOMATO MOSAIC IN PUERTO RICO

A NEW MOSAIC DISEASE OF TOMATO

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INTRODUCTION

Tomatoes are extensively grown in Puerto Rico for home consumption and for the export trade. A fair idea of their importance is shown by the fact that 1500 acres of tomatoes were planted in the vicinity of Villalba and Jayuya during the four-month crop of 1941–42.

Tomato mosaic is a serious and widespread disease and is responsible, in many cases, for great losses. The nature of the causal element or elements of tomato mosaic in Puerto Rico has not been ascertained so far. A survey and study of the virus or viruses causing tomato mosaic; conditions under which it becomes serious, methods of transmission, host range, and the reaction of different commercial and non-commercial varieties of tomatoes which are mosaic resistant or tolerant to the virus or viruses encountered, are necessary before attempting to produce commercial varieties of tomatoes adapted to local conditions.

During the summer and fall of 1942, the Agronomy Department of the Agricultural Experiment Station of the University of Puerto Rico was confronted with a mosaic-like disease which rendered useless the tomato seedlings propagated for distribution among farmers. Some of these seedlings planted in the Station propagating garden were severely affected with the disease. The tomato plants found affected were of the varieties Marglobe, King, and Newark.

SYMPTOMS

Under field conditions, during the hot and humid summer and fall months of 1942, the affected tomato plants showed, in their late stages of growth, a characteristic faint yellowish mottling of the leaves with little or no leaf

distortion. Often the mottling was barely perceptible, or it might altogether disappear. Such affected tomato plants apparently grew normal and produced fairly good crops.

Tomato plants found affected early in their development, showed a pronounced retardation in upward growth, with a condensation of the axis, a progressive decrease in leaf size and various forms of leaf deformation. Necrosis of the growing tips developed frequently. Necrosis also occurred in leaves showing marked reduction in size and deformation. The midribs and lateral veins of affected leaves showed, especially on the underside, a peculiar purplish coloration followed by necrosis, the latter extending throughout the leaf forming large, irregular blotches. Eventually when the whole lamina was affected and had disintegrated, the midrib was left bare. The stems of such severely affected plants became heavily streaked with broad, black and short, or narrow, longitudinal streaks of varying lengths. New shoots were produced below the affected parts of the axis and also frequently became equally affected. On new shoots, formation of leaves was almost always reduced to malformed, purplish outgrowths which finally dried up. The flowers of such severely affected plants were commonly malformed and abortive and, if any fruits were produced, they were small and streaked.

Under greenhouse conditions, young, artificially inoculated tomato plants showed the mottling symptoms described above with some slight inward curling of the margins and tips of the leaves. Other tomato plants showed tip blight, bushy growth, and some of them succumbed to infection. Under such conditions young tomato plants produced small, narrow or filiform leathery leaves, with a peculiar bronzing previous to blighting.

MATERIALS AND METHODS

Tomato leaves (1) with mottle symptoms, (2) without mottle symptoms and (3) necrotic leave tissue, free from extraneous material, were taken from naturally infected tomato plants and macerated respectively in sterilized mortars. The individual macerates were immediately expressed through sterile cheesecloth. Each inoculum was prepared by diluting the filtrated juices in nine parts of distilled, sterile water, and used immediately. These macerates will be referred to as extracts (1), (2) and (3) in the course of this paper.

The virus once isolated and identified was kept in pure culture in tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and pepper (*Capsicum frutescens* var. California Wonder). The extracts prepared with leaves of the infected plants were identified by the name of the source plant, i.e., tomato extract, tobacco extract and pepper extract.

All inoculations were performed by rubbing gently the upper side of the leaves of the differential host plants with Mikado No. 5 brushes with bristles cut to ½ inch, dipped, in every instance, in the respective inoculum. The inoculated plants were kept in an insect-free greenhouse. Temperature inside the green house fluctuated from 70°F. during the night to 90°F. at daytime. Relative humidity fluctuated from 30 to 80%.

Differential and tomato host plants used throughout the work were grown in sterilized compost soil in 5-inch pots in insect-free insectaries inside greenhouses. Inoculations were performed when the young plants had developed 3 or 4 pairs of leaves. Inoculated plants were kept growing until flowering to observe their reaction throughout their whole life-span.

EXPERIMENTAL RESULTS

The Causal Agent

Young plants of tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and *N. glutinosa* were respectively inoculated with each of the three virus extracts.

Tomato plants reacted to inoculation with extracts (1) and (2) with systemic mottling and inward curling of the margins and tips of the leaves. The same extracts produced systemic vein clearing, vein banding, and a chlorotic mottling in tobacco. *N. glutinosa* reacted with vein clearing, systemic mottling and chlorosis.

Percent of infection obtained by inoculating with extracts (1) and (2) was very low. None of the host plants utilized reacted to inoculation with extract (3) (Table I).

Absence of local necrotic spots on *N. glutinosa* when inoculated with extracts (1) and (2) discarded the possibility that the mosaic of tomato might be due to *Tobacco Virus 1*, Johnson.

Young cucumber (*Cucumis sativus*) and black-seeded cowpea (*Vigna sinensis*) plants failed to react when inoculated with any of the virus extracts.

In order to determine the possible relation of the virus or virus entities attacking tomatoes to that recently reported in peppers by Roque and Adsuar (6), pepper plants (*Capsicum frutescens* var. Large Bell Hot) were respectively inoculated with tomato, tobacco and *N. glutinosa* virus extracts. Within five to six days the inoculated pepper plants showed the characteristic symptoms described on this host when infected with the pepper virus as reported by these workers, i.e. systemic vein necrosis, stem streak, and finally defoliation and death. *C. frutescens* var. California Wonder, reacted with vein clearing, mottling, and stunted growth.

The physical properties of the tomato virus under study in relation to

mechanical and insect transmission, longevity in vitro, thermal inactivation, and dilution end point, were identical to those already reported for the pepper virus found by Roque and Adsuar (6), demonstrating that the virus found in tomato is identical to their pepper virus.

TABLE I

Reaction of Host Plants to Virus Extracts from Various Parts of Affected Tomato Plants. Plants Inoculated in August 1942 and Kept at All Times Inside the Greenhouse in Insect-Free, Screened Rooms

Host Plant	Virus Source	No. Inoculated	Diseased	First Symptoms	Reaction
				days	
Tomato v. Marglobe	Mottled leaves	20	7	15–20	Faint mottled leaves
	Un-mottled leaves	20	5	15–20	Faint mottled leaves
	Necrotic leaf tissue	20	0	0	Negative
Tobacco v. Virginia	Mottled leaves	20	5	7–8	Vein clearing and faint mottle
	Un-mottled leaves	20	9	7–8	Vein clearing and faint mottle
	Necrotic leaf tissue	20	0	0	Negative
Nicotiana glutinosa	Mottled leaves	20	4	25–30	Faint mottle leaves
	Un-mottled leaves	20	2	25–30	Faint mottle leaves
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. Large Bell Hot	Mottled leaves	20	11	5–6	Vein clearing and necrosis, defoliation and death
	Un-mottled leaves	20	9	5–6	Vein clearing and necrosis, defoliation and death
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. California Wonder	Mottled leaves	20	4	10–12	Systemic leaf chlorosis and deformation
	Un-mottled leaves	20	6	10–12	Systemic leaf chlorosis and deformation
	Necrotic leaf tissue	20	0	0	Negative

Virus Source and Virulence

Infection of tomato and tobacco with virus extracts from affected tomato plants is not so readily obtained as when the virus is taken from *N. tabacum* var. Virginia or from *N. glutinosa*. An increase in virulence seems to result when the virus is passed through these species of tobacco. De-

creased virulence was noticed when the virus was obtained from tomatoes (Table II).

While infection of *N. glutinosa* by inoculation with tomato mottle-leaf extracts is difficult, however, a high percentage of infection was obtained by inoculating *N. glutinosa* with extracts of either affected *N. tabacum* or *N. glutinosa* plants.

The symptoms in *N. tabacum* are more obvious and more severe. Generally *N. tabacum* var. Virginia, showed vein clearing, faint mottling and vein banding without deformation. When inoculated with a virulent tobacco-virus extract the leaves showed deformation and peculiar dark

TABLE II

Virus Source and Virulence. Young Host Plants Kept Inside Insect-Free Greenhouse

Host	Virus Source	Plants Inoculated	Plants Diseased	Symptoms	Reaction
				days	
Tobacco v. Virginia	Tomato	20	8	7–8	Vein clearing, mottle
	Tobacco v. Virginia	20	17	5–7	Vein clearing, mottle
	N. glutinosa	20	15	5–7	Vein clearing, mottle
N. glutinosa	Tomato	20	0	0	Negative
	Tobacco v. Virginia	20	13	7–8	Faint mottle, chlorosis and death
	N. glutinosa	20	15	7–8	Faint mottle, chlorosis and death
Pepper v. Large Bell Hot	Tomato	20	7	5–6	Necrosis, death
	Tobacco v. Virginia	20	20	5–6	Necrosis, death
	N. glutinosa	20	20	5–6	Necrosis, death
Cucumber	Tobacco v. Virginia	20	0	0	Negative
	N. glutinosa	20	0	0	Negative
		20	0	0	

green markings. *N. glutinosa* inoculated with virulent tobacco-virus extracts reacted within five to six days in contrast with twenty or more days when inoculated with attenuated tomato virus extracts, a high percentage of plants becoming infected. Young *N. glutinosa* infected with the virulent virus showed marked systemic chlorosis, blistering and leaf mottling and finally wilting. This wilting reaction is not produced when inoculation is performed with apparently attenuated virus extracts from tomato.

Tomato plants inoculated with tobacco and *N. glutinosa* virus extracts, responded readily with characteristic leaf mottling, puckering and some-

times with leaf deformation and tip blight. The virus entity on tomatoes inoculated with these two virus extracts is consistently recovered by inoculating expressed sap of infected tomatoes on tobacco and *N. glutinosa*. When a more sensitive test is to be performed there is no better indicator than young, actively growing Large Bell Hot pepper plants. This variety of pepper is so sensitive that it has shown the presence of the virus entity in infected old pepper plants, and in *N. tabacum* v. Virginia and *N. glutinosa* which had been previously inoculated with attenuated virus extracts from tomato and old pepper plants. These tobacco plants had shown a mild expression of the disease becoming later symptomless carriers.

DISCUSSION AND CONCLUSIONS

The present studies demonstrate the presence of the pepper virus (Roque and Adsuar) (6) in tomato plants affected with mosaic. The virus causes a characteristic faint, yellowish mottling and downward puckering of tomato leaves. This has been repeatedly verified by inoculating tomato plants with (1) pepper virus obtained from tomato mosaic affected plants, (2) tomato mosaic virus passed through tobacco and *N. glutinosa* and (3) with pure cultures of the pepper virus thru commercial peppers. The identity of the virus has been substantiated in all cases by the severe vein-necrosis reaction it produces in the Large Bell Hot pepper variety.

Necrotic symptoms, in the form of tip blight and streak, were found to be sometimes associated with the faint mottling, yellowing and puckering so characteristic of the pepper virus in tomato. In that respect it can be stated that it has not been possible to reproduce consistently tip blight necrosis when healthy tomato plants were inoculated either with pepper virus extracts obtained directly from tomato or passed through tobacco. Faint mottling, yellowing and puckering were invariably obtained when such inoculations were performed. In a few cases tip blight symptoms appeared in tomato plants inoculated with virus extracts from tomatoes showing tip blight. This reaction was lost in subsequent transfers, only faint mottling, yellowing and puckering of leaves being observed.

It seems likely therefore, that the blight and necrosis sometimes found associated with tomato mosaic is due, either to a single entity, or to its interaction with the pepper mosaic virus in the tomato plant. In any case the failure to artificially reproduce consistently the tip blight symptoms in plants grown in greenhouses might be attributed, among other causes either to ignorance as to its method of transmission or to a very rapid inactivation of the responsible agent "in vitro."

The fact remains however, that tomato tip-blight like viruses very similar to the one described here have been encountered and reported by other investigators. Among those so far described, that responsible for the

Oregon tip blight of tomatoes (4–5) is very similar, at least as to clinical syndrom. Until more is known about the tip blight virus found in Puerto Rico in respect to transmission and physical properties, its nature will remain a mooted question.

The apparent loss of virulence resulting when the tomato mosaic virus is consecutively passed through tomato or submitted to a long sojourn in either tomato or old pepper plants, as well as its increased infectivity when transferred to tobacco, are phenomena already well established for other viruses. Its importance in connection with this or similar work is that it serves to explain the low percentages of infection, or even at times, the failure to transmit the disease when the virus is passed through different hosts.

Failure to recover the virus entity from dried leaves of affected tobacco plants and from necrotic tomato leaves suggest the possibility of control by cutting affected plants and letting them dry "in situ." As the virus is transmitted mechanically, care should be taken not to carry it from affected tomato, tobacco or pepper plants while working in seed beds. Pepper is extensively grown in Puerto Rico and is an important virus source.

RESUMEN EN ESPAÑOL

Se confirma la existencia en Puerto Rico de un mosaico del tomato causado por el virus del pimiento tal como ha sido informado por Roque y Adsuar (6).

La enfermedad se caracteriza por un matizado del follaje acompañado frecuentemente por una necrosis de las hojas y tallos jóvenes. Esta suele extenderse en estrías negruzcas al largo de los tallos.

Se ha demostrado que el virus pierde su poder infeccioso en tejidos necróticos.

El hecho que los síntomas necróticos no acompañen necesaria y consistentemente al matizado de las hojas, hace pensar que haya mas de un virus envuelto en la compleja manifestación de la enfermedad.

Todo parece indicar que la necrosis que acompaña al matizado de las hojas se deba o bien a una o varias entidades distintas al virus del pimiento, o a la interacción de estas con el virus del pimiento.

Se ha observado una decidida atenuación del virus del pimiento al recuperarse del tomato. Por otro lado se ha comprobado que éste puede recobrar su virulencia al cultivarse en tabaco.

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PLATE A

FIG. 1. Terminal shoot of naturally infected tomato plant showing symptoms of mosaic in the form of leaf deformation, a peculiar purplish coloration of growing tips followed by necrosis and broad, black, short or narrow, longitudinal streaks on stem and branches.

(García and Adsuar: Studies on Tomato Mosaic in Puerto Rico)

ACROTHECIUM LEAF SPOT OF *BASELLA RUBRA* L.

By L. A. ALVAREZ GARCÍA

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Basella rubra L. (*B. alba* L.), known commonly as Malabar Nightshade in Asia and "Espinaca del País" in Puerto Rico, is a succulent annual or biennial vine of Asiatic origin. It is grown in Puerto Rico and in other regions of the Caribbean area for its edible foliage composed of alternate, entire leaves.

At present, there is only a small acreage planted in Puerto Rico. Some few acres are grown to supply public school lunchrooms. Vines are sometimes seen growing on home gardens. This plant can supply a steady and cheap crop of leaves throughout the entire year. The succulent leaves can be used green or cooked, being more appetizing in the latter manner.

The small acreage of this crop in Puerto Rico is to be attributed to the lack of knowledge among farmers of the advantage of having a cheap source of greens for home consumption the year round. It is a well known fact that one of our great dietary problems in Puerto Rico is the insufficiency of roughage in our meals. Vegetable growing in Puerto Rico is a risky proposition and an expensive one for the average farmer. The small farmers could solve in part their roughage problem by a more liberal use of this fast-growing crop. A few plants suffice to take care of the family needs.

A review of the available literature reveals but very few organisms attacking *Basella rubra* L. From Brazil (9), three leaf parasites causing appreciable damage to this plant have been reported: A *Stagonospora*, a *Cercospora* and a *Phyllosticta* species.

In Puerto Rico the writer observed a severe leaf spotting of *B. rubra* among plants grown in a vegetable garden of the W.P.A. (Work Project Administration), located at the Experimental Station grounds, at Río Piedras, P. R. The serious outbreak occurred during the hot, humid summer of 1943.

The disease was characterized by numerous, reddish, subcircular leaf lesions and reddish, elongate vine spots. A parasitic fungus of the genus *Acrothecium* was almost invariably isolated from the red leaf and vine lesions. This organism has never been reported in Puerto Rico nor apparently elsewhere; hence research was conducted to elucidate the host-parasite relationship, under our environment, in the hope of finding a practical measure of control of the disease.

THE DISEASE

The disease caused most serious damage to the foliage, impairing its food value. Primary infections of leaves appeared as a few small yellowish-

brown or reddish spots which enlarged slowly when weather conditions were relatively dry. Leaf spots sometimes showed yellowish, marginal zones, but the affected area remained sharply defined. When the enlargement of the leaf lesions was arrested, a distinctive reddish halo was formed around the innermost, necrotic dirty-brown leaf tissues of the lesions.

During periods of humid atmospheric conditions, the number of leaf and vine lesions was very large, the leaf spots enlarged very rapidly and in many instances the enlarging spots coalesced and formed great patches of necrotic tissues. Such rapidly enlarging spots failed in many occasions to form the characteristic reddish halo observed in lesions with arrested growth.

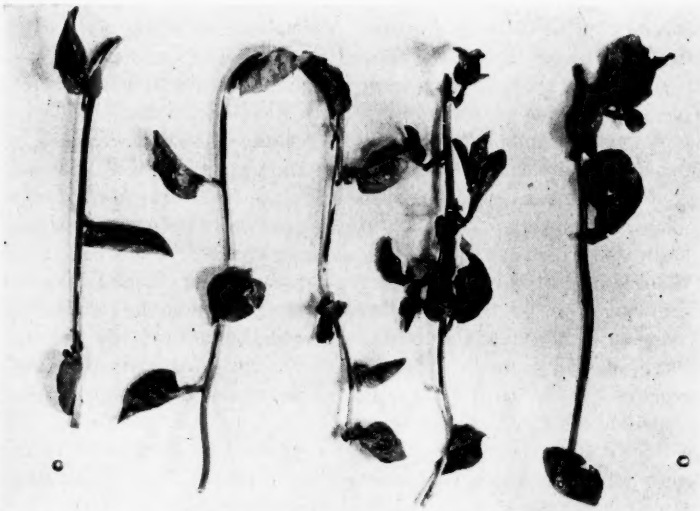


FIG. 1. Natural infections on cuttings of *Basella rubra* L. showing leaf and vine lesions caused by *Acrothecium basellae*. Size greatly reduced.

Leaf lesions were frequently observed developing near the leaf margins, and particularly near the tips. If near each other, the lesions coalesced and enlarged toward the midrib. With the progress of the disease the leaf margins became ragged. Severely affected leaves became chlorotic, the yellowing invading nearby necrotic leaf areas and extending throughout the blade. Such severely infected leaves abscised. Affected leaves showed pronounced curling and sometimes rolling. Young as well as mature leaves were found very susceptible to the disease, although more so in muggy weather (Fig. 1).

Vines of infected plants at first showed very small reddish lesions similar

to those already described occurring on leaves. In dry weather, the lesions enlarged longitudinally, forming long, reddish spots which in very few cases girdled the vines. The lesions were lustrous, reddish and dry, very superficial. The parasite confined its parasitism almost entirely to the endodermal tissues of the vines, except in very muggy weather when the internal vine tissues were also involved. Under such cases of extreme humidity and hot temperature, the vine lesions were also found invaded by secondary soil organisms. A *Rhizoctonia* sp. was frequently isolated from *Acrothecium* lesions at the base of the vines or on vines running over the ground (Fig. 2).

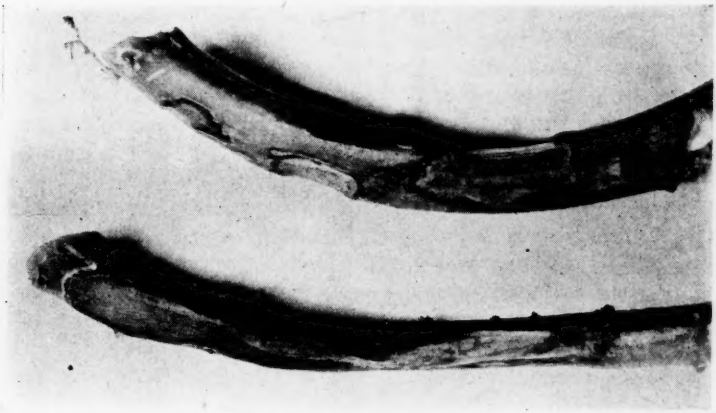


FIG. 2. Advanced lesions at base of vine of *Basella rubra*, caused by *Acrothecium basellae*. Natural size.

The secondary invasion of *Acrothecium* vine lesions by the saprophytic *Rhizoctonia* sp. culminated in a soft, mushy rot of the vines at the points of infection. Eventually the whole vines dried up.

THE FUNGUS

Taxonomy. The parasite is a species of *Acrothecium*, a Deuteromycete belonging to the group Dematiaceae of the Fragmosporeae (Fig. 3). This is the fifth record of an *Acrothecium* from Puerto Rico. In 1919, Tehon (9) described a fungus on *Setaria* sp., as *A. flacatum*. In 1930, Ashford and Ciferri (1) described under the name of *A. obovatum subcapitulatum* an organism growing saprophytically on human skin. Toro, in 1926, described the fungus *A. polytriades* occuring on leaves of *Polytriades amauris* L. *A. capsici* Turconi, was reported on *Capsicum frutescens* L.

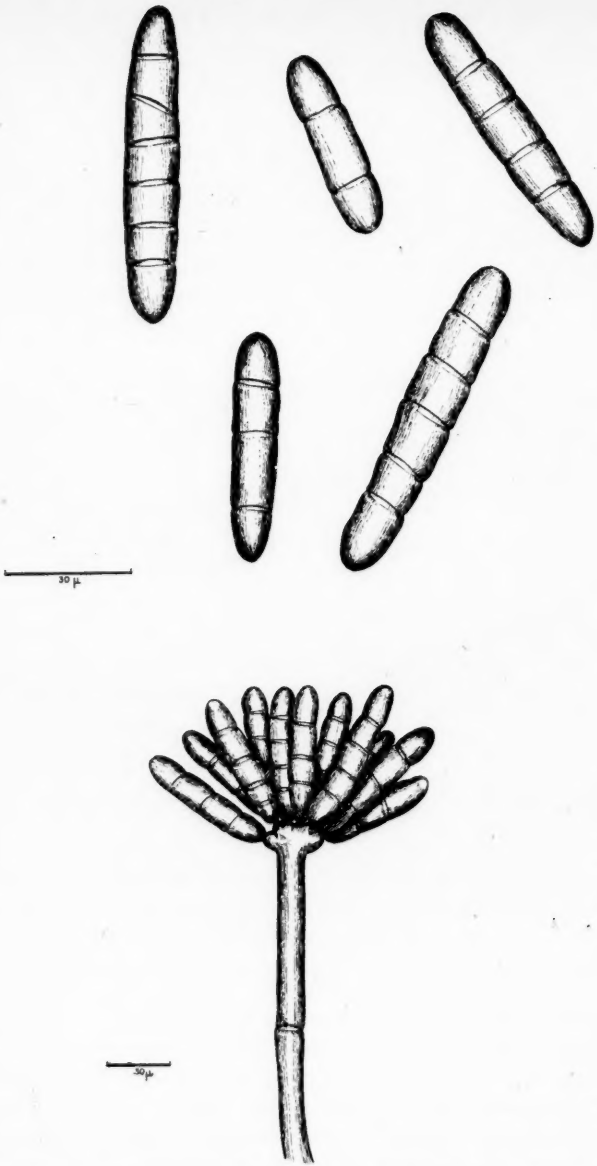


FIG. 3. Conidia of *Acrothecium basellae* and conidiophore the latter showing manner of sporulation. Material obtained from a pure culture of the organism on potato-dextrose-agar kept at 30°C. Drawing made with camara lucida.

Other organisms reported outside Puerto Rico are: *A. nigrum*, *A. obovatum* as a saprophyte on wood, *A. lunatum* Wak., growing saprophytically on sugarcane leaves, also parasitic on *Panicum frumetaceum*, *Eleusine coracana* and *Setaria italica* in India, and *A. penniseti* responsible for a leaf spot of *Pennisetum Typhoideum*. The morphological characters of all these organisms are different from those of the parasite described in this paper, particularly in shape and size of spores and the formation of sclerotia of the latter, a characteristic which appears to be reported here for the first time for this genus.

Many authors have recognized the necessity of revising the genus *Acrothecium*. They substantiate their criteria on the basis of morphological variations of organisms grouped in this genus; variations that participate in characteristics of other genera. Ashford and Ciferri (1) reported variation in cultures of *A. obovatum subcapitulatum* corresponding to such other genera as *Brachysporum*, *Spondylocladium* and *Napicladium*. They also reported that uniseptate conidia resembled *Cordana* and continuous conidia *Acrotheca*.

Tehon (9) found similar behavior in sporulation and other characters in culture of *A. flacatum*. Manoranjan Mitra (5) also observed in cultures of *A. penniseti* the formation of acrogenous conidia and group of conidia below the apex of the conidiophores. *A. basellae*, the parasite on *B. rubra* and the subject of this paper, produced only acrogenous, cylindric spores, conforming in these and other particulars very closely to the general characters ascribed to the genus *Acrothecium*.

Morphology of the fungus on the host: The mycelium consisted of subhyaline hyphae which ramified inside the host tissues, intra and intercellularly, involving the entire leaf tissues. In the vines, the mycelium was confined mostly to the ~~end~~epidermal cells.

Conidiophores were produced amphigenously in the central necrotic tissues of the leaf lesions, arising from sub-stomatal, sclerotia-like bodies or pushing out to the surface between dead cells. In dry conditions, few conidiophores were observed on the leaf lesions, but they were very abundant when the relative humidity of the atmosphere was high. The conidiophores were solitary, rigid or slightly bent, long-septate, very long, sometimes reaching a length of 500 μ or even longer. In width, the conidiophores varied from 9 to 15 μ. The conidiophores were subhyaline when young and not particularly differentiated from the fruiting mycelium. In old cultures, the conidiophores turned brownish. At the apex of the long conidiophores tubercle-like sterigmata were produced in variable numbers, depending on the degree of sporulation which had occurred at the time of microscopic observation. Sterigmata when in large number, formed very conspicuous grape-like clusters. Sterigmata fluctuated from 9 to 15 μ in diameter.

Conidia were produced acrogenously, single or fasciculate as outgrowths from the sterigmata. At first, the conidia were subhyaline projections which enlarged to become long, cylindric, dark, olive-brown spores measuring from 18 to 92 μ in length and from 7.5 to 15 μ in width. The acrogenous conidia were found in variable number, fluctuating from 1 to 15 or perhaps more, arranged in fascicles at the apex of the conidiophores. Due to the lack of mucilage formation at the head of the conidiophores, the conidia detached easily when blowing a soft current of air over the cultures or when floating the conidiophores and their conidia in water. Conidia formation was very profuse in humid conditions. In dry conditions sporulation was not appreciable. Sporulation was easily induced when infected leaves were placed in moist chambers, spores being formed overnight.

Microscopic examination of infected leaves from plants growing in moist environment revealed a profuse mycelial development of the parasite inside the infected leaf tissues. Abundant conidia formation was noticed at the head of numerous subhyaline or dark conidiophores. Sclerotia formation was abundant in the necrotic tissues of the lesions. The sclerotia were dark, semi-hard, composed of anastomosed, thick wall hyphae, were relatively small and not enlarging beyond 1 mm. in diameter.

CULTURES

Pure cultures of the *Acrothecium* sp. were obtained by pouring dilution plates with conidia washed from infected leaves kept in moist chambers; thus inducing abundant sporulation. Owing to the large size of the spores, fishing them out of the cultures to make subcultures was an easy matter.

Germination of conidia in distilled water. Conidia placed in distilled, sterile water germinated readily within an hour or two. Germination tubes were extruded from apical and basal cells as well as from intermediate cells of the spores. One or more germ tubes were produced either from one or from various cells of the spores. The germ tubes grew very rapidly until the spore was disorganized (Fig. 4, *A*).

Morphology of the fungus in cultures. The parasite grew well on different culture media. Nutrient-2% dextrose-agar was found very satisfactory for growth and sporulation. On this medium the mycelial growth was profuse, provided the pH values were around neutrality and the temperature relatively high. Growth of the mycelium was mostly submerged, radial subhyaline at first and later dark (Fig. 4, *B*, *C*). The substratum changed gradually to brownish.

Sclerotia formation was very profuse, and those formed were imbedded in the medium. At the start of their formation the sclerotia were subhyaline, becoming dark brown at maturity; they were almost homogeneous in structure and shade, not smooth, usually single, sometimes conglom-

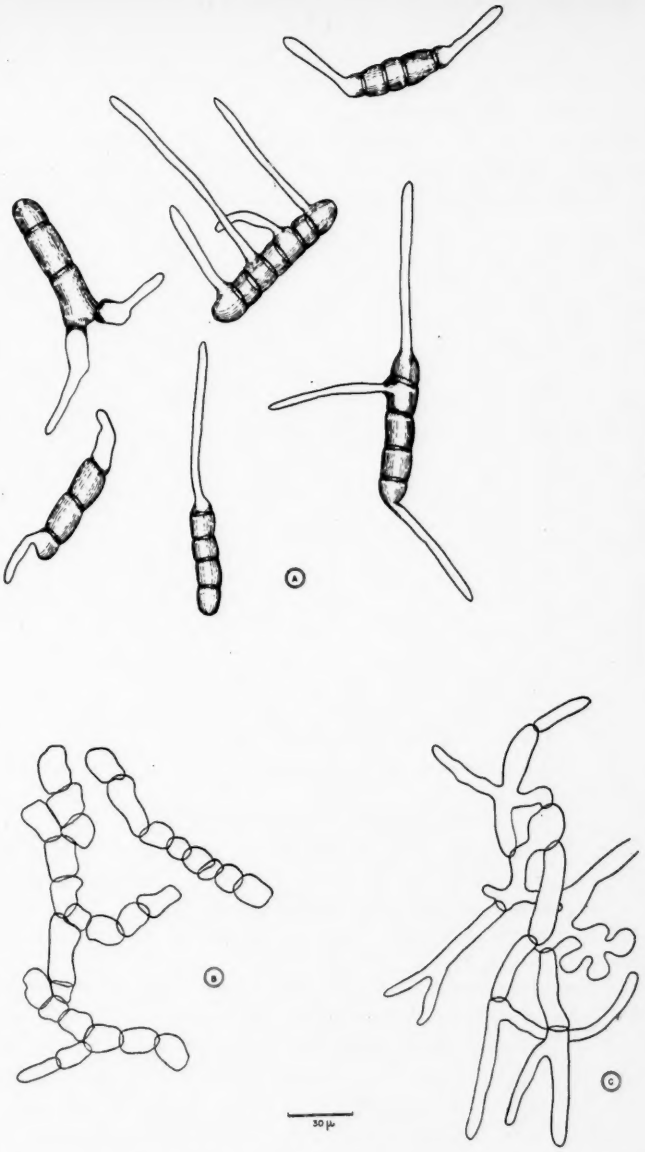
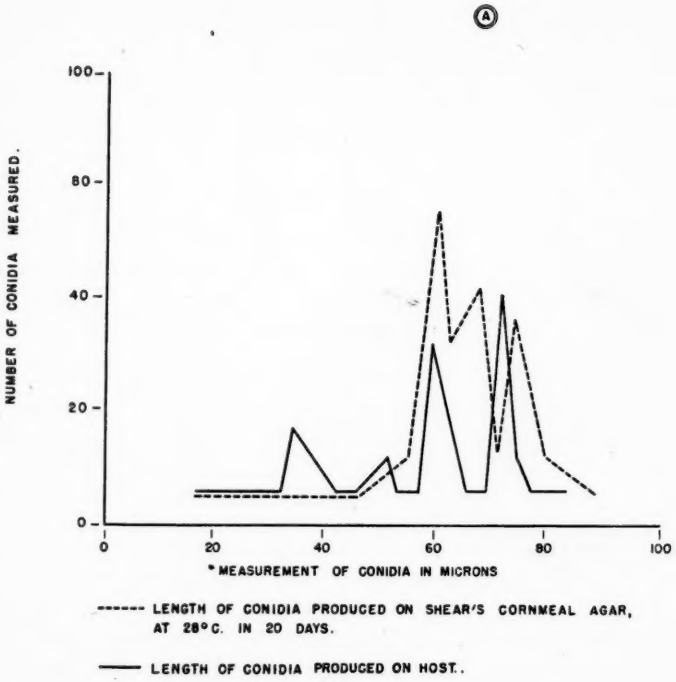


FIG. 4. Above, conidia of *Acrothecium basellae* germinating within three hours of being immersed in distilled water. Below, close-septate and long-septate mycelium of organism in culture.

erated and arranged radially following the growth of the mycelium. Sclerotia fluctuated in size from 160 to 320 μ in diameter, were subspherical or irregularly shaped, semi-hard.

Sporulation was very abundant, conidia being produced apically in fascicle and corresponding in size and number with those observed on natural leaf lesions. Conidia varied from 30 to 72 μ by 9 to 15 μ, were



5A

generally three-septate to five-septate and slightly constricted at the septa. In old cultures the hyphae became thick-walled, forming chlamydospores.

Growth of parasite on other media. On 2% dextrose-agar with an acid reaction of 6.71 and kept at 30°C., the growth was rather poor, superficial, mostly submerged, radial, subhyaline at first, later olive-brown. Few sclerotia were formed, ranging in size from 120 to 320 μ. Sporulation was also poor or did not occur at all in some tubes. Spores were within range of spore measurements observed in nature.

On 2% dextrine-agar, pH 6.37, kept at 30°C., the growth was scanty,

submerged, subhyaline or slightly superficial, turning later olive-brown. Sclerotia varied in size from 108 to 196 μ. Few spores were produced.

On cornmeal (Shear's) agar, pH 6.14, kept at 30°C., the mycelium was submerged or slightly superficial, scattering and radially arranged, closely septate and slightly constricted at the septa. Width of hyphae of submerged mycelium varied from 6 to 9 μ, septation occurred every 15 to 30 μ.

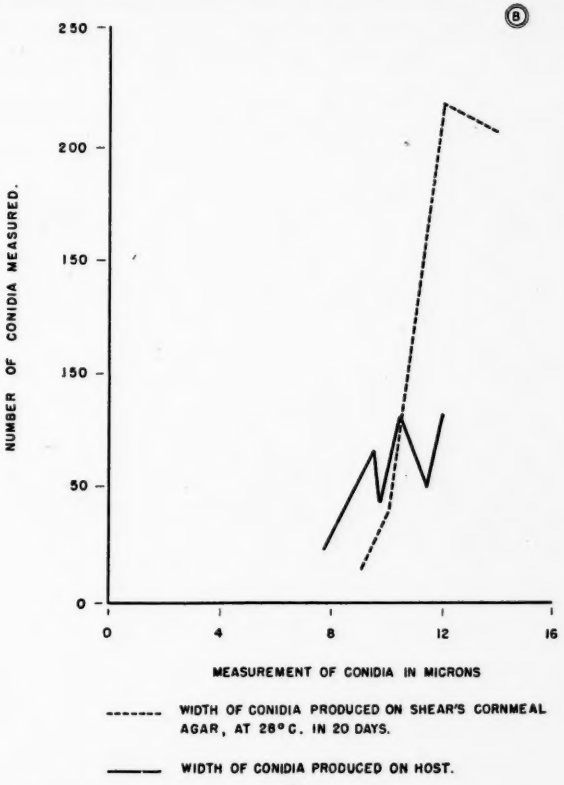


FIG. 5. Comparison of size of spores of *Acrothecium basellae* produced on the host and on artificial substratum.

Conidia formation was relatively abundant; conidia were one to five-septate, granular, and from 24 to 81 μ in length by 9 to 15 μ in width. Conidiophores were very long, slightly tinged brown, not abundant, and rather scattered on the medium. Sclerotia usually were produced rather singly, few in number and relatively small, varying from 45 to 105 μ in size (Fig. 5).

On 2% maltose-agar, pH 5.64, kept at 30°C., mycelial growth was slow, submerged and olive-brown. Spore formation was poor. Sclerotia were abundant, agglomerated, rather small.

Relation of pH values of the substratum and growth. Several lots of 2% dextrose-nutrient-agar plates from the same batch, were treated with lactic acid and adjusted to varying pH values. A range from pH 4.27 to pH 7.15 was prepared; the reactions were determined by means of a potentiometer. Four plates of each set of pH values were respectively planted with mycelial disks, 0.5 cm. in diameter, of the same age. Twenty four hours

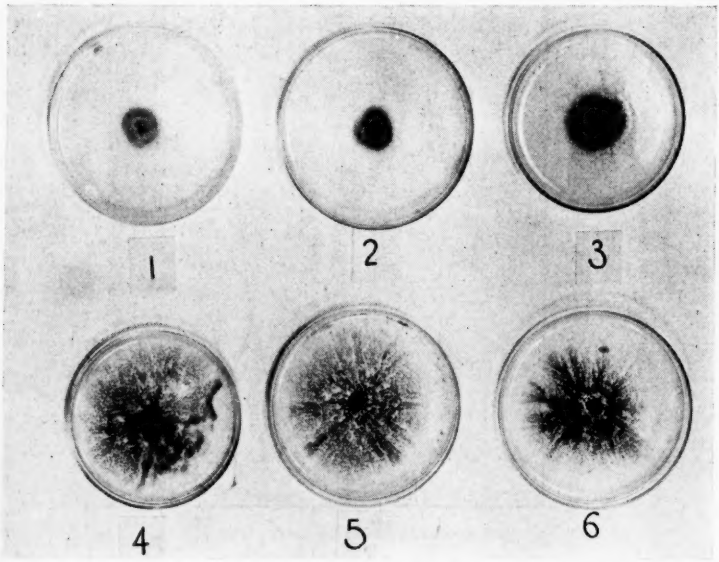


FIG. 6. Growth of *Acrothecium basellae* in six days on dextrose-peptone-agar adjusted to pH values of 4.27, 4.61, 5.29, 5.97, 6.47, 7.15, respectively. Cultures kept at 30°C. Note abundance of sclerotia in plates 4, 5, and 6.

increments of growth of each colony were recorded for a period of seven days. The records represented the average of two diameters of each colony taken at right angles to each other. The average of each set of four plates was jotted down as the growth rate at a given pH value. The results obtained showed that the parasite was favored by slight acid or alkaline reaction of the substratum (Fig. 6).

Relation of temperature and growth. Four plates containing 2% dextrose-nutrient-agar, pH 7.15, were respectively planted with 0.5 cm. in D. mycelial disks taken from young, actively growing cultures of the parasite.

The plates were kept for twelve hours at 28°C., until the colonies have enlarged 2 mm. in radial growth. The cultures were subsequently placed in lots of four in incubators set at 0, 9, 20, 24, 28, 32, 35 and 37°C. The growth rate per day of each colony was recorded as previously explained. The results obtained indicated that high temperatures favored the growth

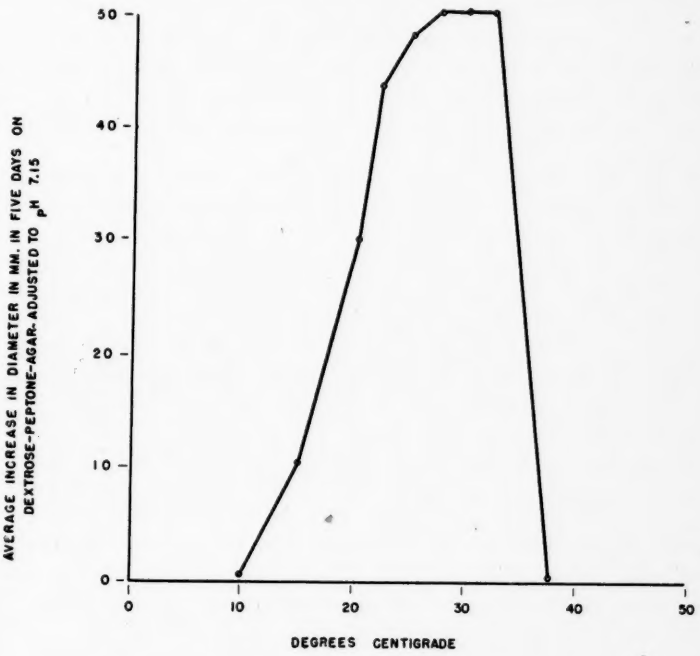


FIG. 7. Relation of temperature to growth of *Acrothecium basellae*

of the parasite. The fastest growth occurred at temperatures within 28 to 32°C. (Fig. 7).

In view of the fact that a parasite of the genus *Acrothecium* attacking *Basella rubra* L. has not been previously reported in American and probably not in Eurasian and African literature, the present fungus is described here as a new species:

Acrothecium basellae sp. nov.:

Conidiophores amphigenous, rigid, erect or subdecumbent, simple, 3 to 5 septate, solitary or sometimes fasciculate; apex swollen with numerous tubercle-like sterig-

mata, subhyaline at first, later olive-brown, not differentiated from the fertile hyphae, except for its long septation and swollen head, length of conidiophores more or less 500 μ. Conidia acrogenous, cylindric, olive-brown, single, produced as an outgrowth of sterigmata, numerous, in groups of up to 15 or more, at apex of conidiophores, easily detached, 1 to 7 septate, slightly constricted at septa, varying in length from 18 to 92 μ by 7.5 to 15 μ in width, thick-walled. Sclerotia numerous in necrotic tissues of leaves and vines, subepidermal, imbedded in substrata, varying in diameter from 40 to 1000 μ, irregularly shaped, dark, semi-hard, not smooth.

PATHOGENICITY

Observations of artificial inoculation with conidia obtained from pure cultures of the parasite and from natural infections on leaves, followed by reisolations of the parasite, showed the constant association of the *Acrothecium* sp. in question and the disease.

Inoculations were conducted in a greenhouse with an air temperature fluctuating from 24°C. to 37°C., and outside under a half lath-shade plant house with an air temperature ranging from 26 to 30°C. Cuttings of *B. rubra* apparently free from natural infection, were obtained and rinsed several times under a faucet to remove dirt and possible conidia of the parasite that might have landed on them. The cuttings were placed with cut ends immersed in distilled water and in a three-salt nutrient solution. In this manner they were left for four weeks in a humid environment to allow for germination of conidia of the parasite if present on the leaves, and infection.

In August, the disease-free cuttings having several leaves, were inoculated with a suspension of conidia of the parasite taken from pure cultures of the organism. The conidia were sprayed over the leaves and vines of the cuttings with a De Vries atomizer. The cuttings after spraying were kept inside a large glass box with adjustable side doors and with inside shelves for keeping a thermograph and a hygrograph. The air temperature inside the glass box fluctuated from 26 to 35°C. daily and the relative humidity was around 80%. Control plants were similarly treated but not inoculated, spraying being done with distilled water only. Twelve cuttings were used in this test. A parallel test was conducted outside under the half-shade plant house.

Within a week after inoculation, the cuttings kept in the glass box showed symptoms of disease. Small yellowish lesions were evident on the leaves (Fig. 8). Seven days later, small reddish lesions were also evident on the vines. Cuttings kept outside under the lath-shade plant house were also showing the same symptoms of disease. The weather outside has been rather hot, 30°C. in the shade, and the relative humidity very high, sometimes around 80%, due to the frequency of rains and numerous cloudy days.

After infection was noticed on the inoculated cuttings, half of them were taken out of the glass box and kept in a greenhouse with a relative humidity around 40%, and a temperature range of 26°C. to 35°C. daily.

Observations of the infected cuttings kept inside the glass box and those in the greenhouse, showed that relative humidity of the environment is the most important factor for disease. The cuttings in the glass box showed constant enlargement of the leaf and vine lesions, culminating in blight. Cuttings outside the box in the greenhouse showed arrested development of lesions and a complete check of further infection and blight. It appeared therefore that under natural conditions the organism isolated and found parasitic on *B. rubra*, is favored by high relative humidity and high temperature. During the summer of 1943, the weather was hot and humid

FIG. 8. Artificially infected leaves of *Basella rubra* L., showing characteristic lesions produced by *Acrothecium basellae*. Uninoculated leaf at left. × ½.

conditions which undoubtedly favored the rapid spread of the disease. Abundant precipitation occurred and the temperature was high, the days were cloudy and muggy.

Plants in the WPA garden were grown on trellis and in an exposed place, which though windy, was particularly humid due to the humid air coming from nearby hills. In this environment very copious sporulation occurred as shown by periodic microscopic examination of leaves. Periodic examination of recently infected leaves also revealed the manner of fungal penetration of healthy tissues. Penetration was observed to occur either through the cells or between the cells or by way of stomata. In some of the germinating spores, appresoria-like structures were noticed before penetrating pecks were produced.

Conidia were evidently blown from place to place. Infections occurred

more abundantly on the leeward side of the field. Leaf lesions were very abundant in mature and in newly formed leaves.

Microscopic examination of infected leaves that had fallen on the ground revealed the profuseness of sclerotia formation in the dead leaf tissues. It appears, therefore, that the parasite is able to pass its saprogenic cycle in the soil in the form of sclerotia. With the advent of favorable weather conditions these sclerotia are liable to germinate and sporulate. When dead leaves with abundant sclerotia were placed in moist chambers the latter were found to germinate. The mycelial growth sporulated in few days. This tends to confirm the supposition that saprogenesis occurs in nature in the form of sclerotia.

CONTROL

The value of spraying with Bordeaux was determined before attempting to try other fungicides. Weekly spraying with Bordeaux formulae 3–3–50 and 4–4–50, proved very satisfactory in controlling the disease. Best results were obtained with the stronger formula. Spraying was initiated on September and continued until November. The summer was very humid and hot, characterized by frequent rainy and cloudy days.

One row of plants, approximately 24 feet long was selected on the leeward side of the infected field. In this manner there was a good chance for a constant blowing of conidia from the infected windward side of the field. At the end of eight weeks of weekly sprayings new shoots and leaves were produced almost free from infection. Unsprayed plants left as control on the same row, showed abundant leaf and vine lesions.

The disease was found to be checked by dry spells. Relative humidity is considered the most important factor determining the incidence of the disease in Puerto Rico, where high temperatures are registered the year round. Therefore, spraying was necessary only during humid weather. Plowing under infected plant debris, and crop rotation are also recommendations which might help in the prevention of the disease.

SUMMARY

A leaf spot of *Basella rubra* L., caused by an *Acrothecium* species, not previously recognized in Puerto Rico nor probably elsewhere as a parasite of the above mentioned plant, is described in this paper. The parasite was found to produce leaf lesions within 5 to 7 days after inoculation with conidia obtained from pure cultures of the organism. Vine lesions were somewhat delayed in their appearance.

Humid atmospheric condition accompanied by relatively high temperature, favored the incidence of the disease, as shown by pathogenicity tests conducted under glass house and field conditions. The foliage of heavily

infected plants blighted in humid environment and became useless for consumption.

Weekly sprayings with Bordeaux, 3–3–50 and 4–4–50, were found successful in controlling the disease, the latter formula giving better results under persistent humid conditions of the environment.

RESUMEN EN ESPAÑOL

Durante el verano del 1943 se observó en una siembra de Espinacas del País (*Basella rubra* L.) una chamusquina de las hojas, apareciendo ésta primeramente en forma de pequeñas manchas rojizas que al agrandarse y venir en contacto formaban mancharones de tejidos necróticos descoloridos.

El ambiente en que crecían dichas plantas demostró ser propicio para el desarrollo de la enfermedad. El tiempo fué húmedo, cálido y los días se presentaron lluviosos y generalmente nublados. Un examen microscópico de las lesiones en las hojas reveló la abundancia de conidióforos extremadamente largos y rematados en esterígmatas en forma de ramilletes de uvas. De estas esterígmatas produjéronse esporas alargadas, cilíndricas, tabicadas hasta 7 veces, trasversalmente, y formando en conjunto un ramillete. La morfología de este organismo demostró pertenecer al género *Acrothecium*. Por considerarse una especie hasta el presente no informada en *B. rubra*, se describe en este trabajo como una especie nueva, dándosele el nombre de *Acrothecium basellae*.

Al hacerse cultivos con esporas tomadas de lesiones en las hojas, como así también de esclerocios tomados de tejidos necróticos en dichas hojas, se aisló invariablemente el susodicho organismo. Pruebas de patogenicidad realizadas con conidios del organismo producidos en cultivo puro demonstraron incontrovertiblemente su virulencia y única relación con la enfermedad en cuestión. Los síntomas producidos fueron aquéllos anteriormente observados ocurriendo naturalmente en las hojas. El examen microscópico de las lesiones producidas por inoculación artificial con conidios tomados de cultivos puros, reveló la presencia del organismo en los tejidos parasitados y la morfología del mismo vino a corroborar su identidad como el que fuera aislado repetidamente de lesiones naturales en las hojas. Al hacerse cultivos de tejidos de lesiones de hojas artificialmente inoculadas se recuperó invariablemente el parásito.

El examen necroscópico de las lesiones en las hojas mostró la presencia de abundantes esclerocios en los tejidos subepidermales. Las observaciones hechas hacen creer que el parásito perdura saprogénicamente en residuos de hojas y tallos enfermos. Cuando el ambiente es propicio germinan dichos esclerocios; producen nuevamente conidios y la enfermedad vuelve a aparecer.

Ensayos realizados con caldo bordelés (fórmulas 3–3–50 y 4–4–50)

demostraron que la enfermedad se puede combatir efectivamente con este fungicida. La fórmula más concentrada dió mejores resultados, particularmente en tiempo extremadamente húmedo. Las pulverizaciones se espaciaron cada 7 días, encontrándose que así repartidas eran suficientes para combatir la enfermedad.

Pruebas hechas en invernáculos demostraron como, en un ambiente marcadamente seco (40% de humedad relativa), se retardaba marcadamente el aumento en tamaño de las lesiones y disminuía el número de éstas. En tiempo marcadamente seco no es de esperarse que sea tan severa la enfermedad; bastaría, si fuere necesario, pulverizar con bordelés de vez en cuando.

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ALTERNARIA BLIGHT OF BACHELOR'S BUTTON (*GOMPHRENA GLOBOSA* L.)

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During the hot, humid summer of 1937, the writer observed a severe case of leaf and stem blight of the ornamental plant commonly known as Bachelor's Button (*Gomphrena globosa* L.), in flower gardens in the neighborhood of Río Piedras. Outbreaks of the blight were later observed throughout the Island. In every instance an *Alternaria* species was isolated from affected leaf and stem tissues of diseased plants collected in flower gardens. A similar fungus was also isolated from the wild species *G. dispersa* Standley. Pathogenicity test with these fungi showed that both organisms were one and the same species. The purple, white and red varieties of *G. globosa* were found to be equally susceptible to the disease.

Up to the present and to the author's knowledge, there has never been reported in Puerto Rico a fungus of the genus *Alternaria* attacking *Gomphrena* species. Apparently there is no record of such occurrence in American literature.

In Japan, Togashii (1) reported a severe blight of *G. globosa* and showed by artificial inoculation with conidia of an *Alternaria* species, that this fungus was the causal agent of the disease. No infection, however, was obtained when inoculating with conidia obtained from pure cultures of the fungus. Yoshii (2) also reported a similar organism, resembling morphologically *A. gomphrenae* Togashii responsible for a leaf spot of *G. globosa*. The organism however, is claimed to be different to that previously reported by Togashii as far as cultural characters are concerned. Both species of *Alternaria* produced similar clinical syndroms, characterized by dark reddish purple spots with smoky-gray necrotic centers.

The leaf symptoms of the *Alternaria* blight observed in Puerto Rico correspond with those described in Japan. The morphology and physiology of the *Alternaria* species prevalent in Puerto Rico seems to justify our assumption that this fungus is the same species already reported and described in Japan by Togashii.

THE DISEASE

The first noticeable symptoms of the disease under our environmental conditions were the appearance of small (0.25–0.50 mm. in diameter) yellowish-green spots, visible on both sides of the leaves, although more conspicuously on the upper side. These spots were produced more rapidly

and abundantly on the lower leaves. As the spots enlarged and grew older, they turned yellowish-brown, forming concentric rings of varying shades of that color. The innermost necrotic tissues became discolored and translucent. The invaded tissues around the yellowish-green spots turned first dull green and with the advance of the disease became discolored. When the lesions seemed to have finished their enlargement, a reddish halo was formed encircling the yellowish-brown spot. This halo was characteristic of the disease and did not fade out except when the lesions were very old and the leaf had turned light brown. In some spots, for some reason, the halo was not formed or was very pale. In old lesions although the halo had vanished, the affected necrotic areas were sharply defined. The spots appeared scattered all over the leaf surfaces and were of a subcircular outline, except when they occurred near the margins or near the veins, in which case they became irregular. Nearby spots enlarged, coalesced, and envolved a great part of the leaf. With the progress of the disease, the leaves corrugated and rolled, becoming finally dirty brown throughout and were found hanging down along the stems. They stayed in this position for several days and then dropped. The lesions on the stems were very similar to those found on the leaves. However, on the stems they were elongated. Some of the stem lesions on becoming old lost their dahlia-carmine halo and a whitish discolored area remained. These lesions enlarged, coalesced and girdled the plant.

THE PATHOGENE

Methods of isolation. During the summer of 1937, the fungus was isolated from fresh, young lesions on leaves and stems of the purple variety of *G. globosa* L., by planting pieces of diseased tissues, previously disinfected for 2–3 minutes in a 1–1,000 solution of bichloride of mercury and afterwards washed several times in sterilized water, on agar plates and from dilution plates of conidia washed from young lesions.

The organism in culture. The fungus grew well in potato-dextrose agar, prune agar, bean agar, Cook's No. 2 agar, and oatmeal agar. In oatmeal agar, the organism formed aerial, cottony, white to light gray mycelium and olive-brown or ochreous trophic and fructiferous growth. The nonfertile hyphae were elongate-septate, minutely guttulate and with septa spaced from 25 to 83 μ and slightly constricted. Fertile hyphae were recognized by the toruloideous sub-fasciculate shape, by the presence of large, refractive oil globules which in some instances filled the lumen of the cell and by the closeness of the septa, spaced from 3.6 to 7.2 μ. These fertile hyphae were definitely light brown or ochreous in color, and were found to produce numerous chlamydospore like bodies. The substratum of the media and particularly those with a high content of carbohydrates

was tinged dark brown or ochreous. Sporulation occurred on oatmeal agar and in small amount. The conidiophores as well as the conidia were formed from protuberances of the cells and were hyaline at first. Later, at maturity, they turned light-brown or ochreous. No sporulation took place in other culture media.

Morphology on the host. The fungus once it had entered the host, ramified and spread in the host tissues. The hyaline hypha, of approximately 8 μ in thickness, was septate and slightly constricted at the septa. Eventually, the invaded tissues were killed, became necrotic, and of a yellowish-brown color. The conidiophores were produced amphigenously, although more abundantly on the upper side of the leaf, and particularly along the yellowish-brown area next to the dahlia-carmine halo. These conidiophores were produced singly or in thin tufts, erect or subdecumbent; were 1 to 5 septate and from 35 to 70 by 4 to 5 μ in width and emerged by way of the stomata or pushed their way through the necrotic areas of the lesions. The conidia, formed generally singly at the apex of the conidiophores, were easily detached, being elongate, obclavate, with basal and blunt or pointed, 1 to 9 septate, and slightly constricted at the septa; longitudinal septa few or lacking, granular or with refractive oil globules, 60 to 160 by 12 to 16 μ, long beaked, never branched, septated every 10 to 15 μ and ranged from one to three times the length of the broadest part of the spore.

PATHOGENICITY

Plants grown from seeds in sterilized soil were inoculated on August 8, 1937 in the afternoon of a cloudy day with a suspension of spores washed from the typical lesions and from pure culture, respectively. Two sets of plants were inoculated by means of a DeVries atomizer. No lesions were made to the leaves, stems or branches. Half of the plants from each group were covered with bell jars and the others were left uncovered. Control plants were similarly treated but not inoculated. Three days later, typical tiny yellowish green, spots were evident. Microscopic examination of the matured lesions showed the constant association of the pathogene. Re-isolations from the newly formed lesions ratified the pathogenicity of the organism. Conidia were found to be produced within 4 to 6 days after inoculation.

EPIPHYTOLOGY

High temperature combined with ample atmospheric moisture seemed to favor the development of the parasite. Under such environmental conditions spores were produced abundantly on leaf lesions. Spores are spattered by rain drops or carried by wind. New cases of *Alternaria* blight appeared on plants where the spores have landed. Saprogenesis appeared to occur in debris of diseased plants.

CONTROL

Eradication of dead or severely diseases plants, as well as the destruction of all diseased material by deep plowing or by burning, clean cultivation, good drainage and planting during the dry season will contribute in checking the disease.

SUMMARY

1. An *Alternaria* sp. is reported occurring on *Gomphrena globosa* L. and *G. dispersa* Standley.

2. All the ornamental varieties as well as the wild type are susceptible to the disease.

3. The disease is recognized by the sub-circular, yellowish-brown lesions on stems and leaves with a characteristic reddish halo.

4. The pathogene, an *Alternaria* sp. is described.

5. Conidia from young lesions as well as from an oatmeal culture were used for morphological, physiological and inoculation purposes.

6. The inoculation tests with spore suspensions taken respectively from young lesions as well as from culture proved the pathogenicity of the organism.

7. Conidia placed in water and at room temperature (28 C.) started germination within an interval of 2 to 3 hours.

8. Sporulation was noticed on lesions on the leaves and never on the stems. Dead parts of the plants seem to harbor the pathogene.

9. The disease is more severe during hot, rainy weather.

RESUMEN EN ESPAÑOL

Durante el verano de 1937, especialmente en la vecindad de Río Piedras, se pudo observar la difusión de una enfermedad atacando las hojas y las ramas de las siemprevivas (*Gomphrena globosa* L. y *G. dispersa* Standley). Se presenta esta enfermedad en las hojas inicialmente con manchas esparcidas, rara vez confluentes, pequeñas de 0.25 a 0.50 mm. de diámetro, circulares, amarillentas y bordeadas por una zona verde mate. Las lesiones al aumentar de tamaño pueden ser irregulares, confluentes y presentan los tejidos centrales necróticos y de color pardo claro y rodeados muy claramente por un área de color rojizo, persistente. Las hojas atacadas se deforman, mueren, cayéndose finalmente. En los tallos las lesiones muestran características similares, pero son generalmente irregulares, alargadas y unilaterales, pero en ciertas ocasiones son confluentes, rodeando el tallo y produciendo la muerte de la parte superior.

Un examen microscópico de las lesiones muestra los tejidos serpenteados por el micelio tabicado de un hongo de donde emergen, sea a través de los

tejidos o por estomas, los hacecillos de conidioforos simples, obscuros, contínuos o tabicados, al extremo de los cuales se forman esporas mazudas, oliváceas y acres, tabicadas longitudinal y transversalmente, lo que cataloga a este organismo patógeno entre los hongos del género *Alternaria*. Siendo esta especie un nuevo record para Puerto Rico, se describe a continuación:

Alternaria gomphrenae Togashii. En nuestro ambiente este hongo produce conidióforos obscuros, tabicados una o cinco veces transversalmente, solitarios o fasciculados, erguidos o inclinados, simples o ramosos. Los conidios oliváceos u ocres, mazudos, con el ápice extremadamente alargado y fino, con 1 ó 9 tabiques transversales y con o ningún tabique longitudinal; el contenido granular u oleaginoso, de 60 a 160 por 12 a 16 μ. en tamaño.

La enfermedad se manifiesta mayormente en épocas de lluvias frecuentes y en sitios donde hay poca aereación y mal avenamiento.

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